Crocidolite Activates NF-κB and MIP-2 Gene Expression in Rat Alveolar Epithelial Cells. Role of Mitochrondrial-Derived Oxidants

Kevin E. Driscoll,[1] Janet M. Carter,[1] Brian W. Howard,[1] Diana Hassenbein,[1] Yvonne M.W. Janssen,[2] and Brooke T. Mossman[2]

[1]Procter & Gamble Pharmaceuticals, Cincinnati, Ohio; [2]University of Vermont, Burlington, Vermont

Nuclear factor kappa B (NF-κB) is a transcription factor that regulates expression of several genes coding for inflammatory and immunoregulatory proteins including the neutrophil chemotactic cytokine MIP-2. In previous studies we found that crocidolite asbestos activates the nuclear translocation of NF-κB as well as MIP-2 gene expression in rat alveolar type II cells. Here we report that both crocidolite-induced NF-κB activation of MIP-2 gene expression can be attenuated by the antioxidant tetramethylthiourea, suggesting the dependence of these responses on oxidative stress. Crocidolite exposure of RLE-TN cells also increased production of H_2O_2, a response that was inhibited by the mitochondrial respiratory chain inhibitor thenoyltrifluoroacetone (TTFA). TTFA treatment of RLE-6TN cells also inhibited crocidolite-induced nuclear translocation of NF-κB and MIP-2 gene expression. These results indicate crocidolite exposure of rat alveolar type II cells results in increased production of mitochrondrial-derived hydrogen peroxide and that mitochrondrial-derived oxidants contribute to crocidolite activation of NF-κB and increases in MIP-2 gene expression. — *Environ Health Perspect* 106(Suppl 5):1171–1174 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-5/1171-1174driscoll/abstract.html*

Key words: chemokines, nuclear factor kappa B, crocidolite, macrophage inflammatory protein 2, mitochondria, epithelial cell

Introduction

Occupational exposure to crocidolite asbestos has been associated with development of chronic interstitial lung disease, lung cancer, and mesothelioma (*1–3*). As with a number pneumotoxic agents, the alterations in lung structure and function arising from crocidolite exposure result, at least in part, from the recruitment and activation of inflammatory cells. In this respect, studies using animal models of asbestosis have demonstrated that pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1 contribute to the crocidolite-induced pulmonary inflammation (*4–6*). More recently, *in vitro* and *in vivo* studies have demonstrated that potent leukocyte chemotactic cytokines known as chemokines are mediators of crocidolite-induced inflammation (*4,7,8*).

Macrophage inflammatory protein 2 (MIP-2) is an 8-kDa, heparin-binding protein first identified as a product of endotoxin-stimulated mouse macrophages (*9*). MIP-2 is a member of the chemokine family and is selectively chemotactic for neutrophils and mitogenic for alveolar epithelial cells (*10*). Murine MIP-2 is functionally and structurally related to the human gro chemokines (*10*). Exposure of rats to a variety of particles including crocidolite and quartz under conditions eliciting pulmonary inflammation results in increased MIP-2 expression in the lung (*5,8,10*). Sources of MIP-2 in the rat lung after particle exposure include both the alveolar macrophage and lung epithelial cells (*8*). Regarding the latter, *in vitro* studies have demonstrated that crocidolite can directly activate alveolar type II cells to express the chemokines MIP-2 and IL-8 (*8,11*).

Although the mechanisms by which crocidolite activates rat lung epithelial cell MIP-2 production are unknown, expression of the MIP-2 gene is regulated by the transcription factor nuclear factor kappa B (NF-κB) (*12*). Studies in several different systems indicate the signaling pathways regulating NF-κB involve an oxidant component, although the nature of the oxidant and the mechanism by which it is coupled to the signaling cascade remains undefined (*13*). To better understand the mechanisms by which crocidolite activates MIP-2 gene expression and activation of NF-κB, the present studies investigated the contribution of oxidative stress to crocidolite-induced activation of NF-κB and MIP-2 gene expression; and the potential source of oxidants contributing to this response.

Methods

Culture and *in Vitro* Exposure of Rat Alveolar Epithelial Cells

Epithelial cell responses to particles were characterized using the rat alveolar type II cell line, RLE-6TN (*14*). This cell line retains characteristics of alveolar type II cells, including expression of chemokines after exposure to various agonists (*8,14*). RLE-6TN cells were maintained in 25-cm^2 tissue culture flasks and grown in RluE media (BRFF, Ijamsville, MD). The effects of the oxygen radical scavenger tetramethylthiourea (TMTU) or the mitochondrial respiratory chain inhibitor thenoyltrifluoroacetone (TTFA) on *in vitro* crocidolite activation of nuclear translocation of NF-κB and MIP-2 gene expression were examined using confluent cultures of RLE-6TN cells exposed under serum-free conditions to crocidolite (median diameter [± GSD (geometric standard deviation)] = 0.28 ± 1.8; length [% < 10 μm] = 23; surface area = 3.2 m^2/g) at 10 μg particles/cm^2 of the culture dish for a period of 6 hr. A dose of 10 μg/cm^2 was used because previous studies in our laboratory demonstrated this dose was

This paper is based on a presentation at the Second International Meeting on Oxygen/Nitrogen Radicals and Cellular Injury held 7–10 September 1997 in Durham, North Carolina. Manuscript received at *EHP* 19 February 1998; accepted 9 June 1998.

Address correspondence to K.E. Driscoll, Procter & Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason Montgomery Road, PO Box 8006, Cincinnati, OH 45040-8006. Telephone: (513) 627-2360. Fax: (513) 627-0400. E-mail: driscollke@pg.com

Abbreviations used: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSD, geometric standard deviation; IL, interleukin; MIP-2, macrophage inflammatory protein 2; NF-κB, nuclear factor kappa B; PCR, polymerase chain reaction; TMTU, tetramethylthiourea; TNF-α, tumor necrosis factor α; TTFA, thenoyltrifluoroacetone.

effective at activating these responses in RLE-6TN cells (*8,15*).

Electrophoretic Mobility Shift Assay

The electrophoretic mobility shift assay was used to characterize NF-κB-binding activity as described previously (*16*). Briefly, RLE-6TN cells were rinsed 2 times with cold phosphate-buffered saline, pH 7.2, and the nuclear extracts collected, as described by Staal et al. (*17*). Cells were lysed in hypotonic buffer, 0.6% Nonidet P-40 was added, and lysates were vortexed for 15 sec. Nuclei were pelleted by centrifugation at 4°C. Nuclear protein extracts (4 μg) were incubated for 20 min in DNA-binding buffer (40 nM HEPES buffer, 4% Ficoll 400, 200 ng polyI:polyC per μl, 1 mM $MgCl_2$, 0.1 mM dithiothreitol, and 0.175 pmol ^{32}P-end-labeled double-stranded oligonucleotide containing a consensus NF-κB site [Promega, Madison, WI]). Samples were loaded onto a 5% polyacrylamide gel and electrophoresed in 0.25×Tris borate–EDTA buffer for 2 hr at 120 V. Gels were dried and visualized by exposure to Kodak X-Omatfilm.

Reverse Transcriptase–Polymerase Chain Reaction Analysis of Chemokine Expression

MIP-2 mRNA transcript levels were assessed by polymerase chain reaction (PCR) amplification of the MIP-2 cDNA, as described in detail elsewhere (*10*). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was evaluated as an internal control. The primers were designed from the published sequences for MIP-2 (*18*) and GAPDH (*19*) and were as follows: MIP-2: 5′-GGCACATCAGG TACGATCCAG-3′;5′ACCCTGC CAAGGGTTGACTTC-3′; GAPDH: 5′-CAGGATGCATTGCTGACAATC-3′; 5′-GGTCGGTGTGAACGGATTTG-3′

PCR reactions were overlaid with mineral oil and amplification carried out through 22 to 30 cycles of denaturation at 94°C for 1 min, oligo annealing at 55°C for 1 min, and extension at 72°C for 2 min. Reactions were electrophoresed in 1.5% agarose gels containing ethidium bromide in Tris acetate–EDTA buffer to visualize the MIP-2 and GAPDH PCR products.

Measurement of H_2O_2

To characterize the effects of crocidolite on production of H_2O_2, RLE-6TN cells were cultured at 1×10^5 cells/well in 96-well tissue culture plates (Corning, Corning, NY) and exposed to crocidolite at 10 μg/cm^2 with and without catalase (100 U/ml; Sigma Chemical Co., St. Louis, MO) or the mitochondrial respiratory chain inhibitor TTFA at 200 μM. Hydrogen peroxide in RLE-6TN cell conditioned media was determined after 6 hr of crocidolite exposure based on the horseradish peroxidase-dependent conversion of phenol red by H_2O_2 into a compound with absorbance at 600 nm, as described by Ding et al. (*20*). Briefly, 100 μl phenol red solution (140 mM NaCl, 10 mM KH_2PO_4, 5.5 mM dextrose, 0.56 mM phenol red) (Sigma) containing 6 U/ml type VI horseradish peroxidase (Sigma) was added to cell cultures. After 6 hr at 37°C, 10 μl 1 N NaOH was added and absorbance at 600 nm was determined using an EL311 Biotek Microplate Reader (Biotek Instruments, Winooski, VT). H_2O_2 concentrations were calculated from a standard curve.

Statistical Analysis

Data for H_2O_2 production were analyzed by ANOVA, with group differences determined using the Newman-Keuls test (*21*).

Results

We previously demonstrated that 5 to 20 μg/cm^2 crocidolite activates nuclear translocation of NF-κB in RLE-6TN cells (*15*). To determine if oxidative stress contributed to this response, we exposed cells to 10 μg/cm^2 crocidolite in the presence of the oxygen radical scavenger TMTU, and nuclear translocation of NF-κB was characterized. Crocidolite exposure increased nuclear NF-κB binding activity and treatment of cells with 50 mM, but not 25 mM, TMTU markedly attenuated this response (Figure 1).

To investigate if mitochondrial-derived oxidants might contribute to crocidolite activation of NF-κB, RLE-6TN cells were treated with the respiratory chain complex II inhibitor TTFA and exposed to crocidolite, and nuclear translocation of NF-κB was determined. As shown in Figure 2, TTFA at a concentration of 200 μM, and to a lesser extent 20 μM, attenuated crocidolite-induced activation of NF-κB. We also determined whether crocidolite exposure of RLE-6TN cells resulted in increased H_2O_2 production and if this response was sensitive to TTFA. As shown in Figure 3, exposure of RLE-6TN cells to crocidolite increased the release of H_2O_2 by RLE-6TN cells approximately 1.8-fold (Figure 3). The stimulation of RLE-6TN cell H_2O_2 production by crocidolite was inhibited by TTFA.

In agreement with our earlier findings (*8*), exposure of RLE-6TN cells to crocidolite increased expression of mRNA for the proinflammatory cytokine MIP-2 (Figure 4). Like NF-κB, crocidolite-induced MIP-2 gene expression was attenuated by the antioxidant TMTU and the respiratory chain inhibitor TTFA, indicating a dependence on mitochondrial-derived oxidants.

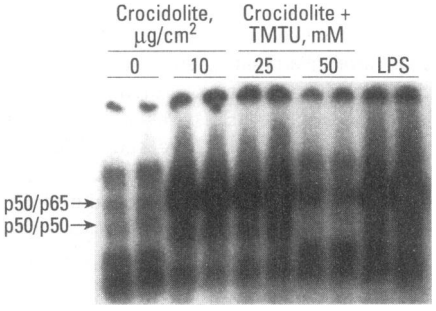

Figure 1. Effect of TMTU on crocidolite-induced increases in nuclear NF-κB binding activity in RLE-6TN cells. Arrows indicate bands demonstrated previously to correspond to the p50/p65 and p50/p50 NF-κB protein complexes (15). Crocidolite exposure of RLE-6TN cells (10 μg/cm^2×6 hr) increased nuclear binding activity for both the p50/p65 and p50/p50 NF-κB protein complexes; lipopolysaccharide (50 ng/ml) also increased nuclear NF-κB binding activity. Treatment of cells with TMTU at 50 mM inhibited crocidolite-induced activation of NF-κB.

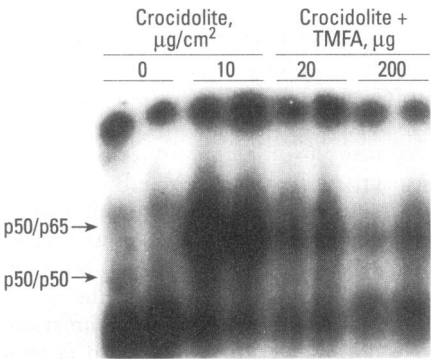

Figure 2. Effect of the mitochondrial respiratory chain inhibitor TTFA on crocidolite-induced increases in nuclear NF-κB binding activity in RLE-6TN cells. Arrows indicate bands demonstrated previously to correspond to the p50/p65 and p50/p50 NF-κB protein complexes (*15*). Crocidolite exposure of RLE-6TN cells (10 μg/cm^2×6 hr) increased nuclear p50/p65 and p50/p50 NF-κB protein complexes. The addition of TTFA at 20 and 200 μM attenuated crocidolite-induced activation of NF-κB.

Discussion

The transcription factor NF-κB can be activated by a variety of pathologic stimuli and regulates the transcription of several genes that contribute to inflammatory processes (*22*). Previous studies have demonstrated that crocidolite can increase nuclear translocation of NF-κB in several cellular targets of asbestos-induced lung disease, including hamster tracheal epithelial cells, rat pleural mesothelial cells, rat alveolar type II cells, and a human lung carcinoma cell line (*15,16,23*). The present studies confirm and extend previous observations by demonstrating that crocidolite exposure of the rat alveolar type II cell line RLE-6TN results in activation of NF-κB, a response associated with increased cellular production of H_2O_2 and inhibited by antioxidants. In addition, this is the first study to show that, like NF-κB, the chemokine MIP-2 is regulated by oxidants and to provide evidence that the source of oxidants contributing to crocidolite activation of and MIP-2 gene expression in rat alveolar type II cells is the mitochondria.

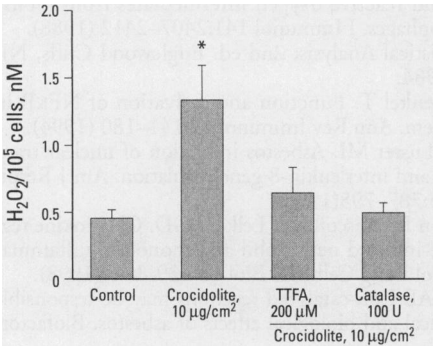

Figure 3. Crocidolite-induced increases in H_2O_2 production by RLE-6TN cells is inhibited by TTFA. Crocidolite exposure of RLE-6TN cells (10 μg/cm² × 6 hr) increased the release of H_2O_2. The addition of TTFA at 200 μM attenuated crocidolite-induced increases in RLE-6N H_2O_2 release, indicating mitochrondria are a source of the oxidant. Asterisk (*) indicates significantly different from control, crocidolite + TTFA, and catalase + catalase groups; $p < 0.05$.

MIP-2 is a key mediator of neutrophil recruitment in the rat lung and plays a role in pulmonary inflammation evoked by a variety of agents, including pneumotoxic particles such as crocidolite and quartz, high lung doses of relatively innocuous materials such as titanium dioxide and carbon black, and the oxidant air pollutant ozone (*5,8,24*). Recent studies indicate that some particles, including crocidolite, can directly activate MIP-2 gene transcription in rat alveolar type II cells and expression of IL-8 mRNA in the human lung carcinoma cell line A549 (*8,23*). Analysis of the proximal promoter region of the MIP-2 gene revealed that it contains a κB site that regulates endotoxin activation of MIP-2 gene transcription in macrophages (*12*). Results of the present studies demonstrate crocidolite-induced expression of MIP-2 in epithelial cells is associated with NF-κB activation and is sensitive to the same factors that inhibit nuclear translocation of NF-κB, suggesting that like endotoxin activation of MIP-2 gene transcription in macrophages, NF-κB contributes to crocidolite-induced of MIP-2 gene transcription in epithelial cells. There is now considerable evidence that reactive oxygen species influence the activation of NF-κB (*13*). Regarding crocidolite activation of RLE-6TN cells, our demonstration that the nuclear translocation of NF-κB and increased MIP-2 mRNA expression can be inhibited by the oxygen radical scavenger TMTU indicates oxidants play a role in these responses. This observation is consistent with recent studies demonstrating that crocidolite-induced increases in nuclear NF-κB in hamster tracheal epithelial cells can be attenuated by treatment with the sulfhydryl reagent *N*-acetylcysteine (*16*). One mechanism by which crocidolite could produce an oxidative stress on cells is through redox reactions catalyzed by metals on the surface of the fibers (*25*). Alternatively, oxidants could be produced as a result of the fibers stimulating production of cell-derived oxidants. As reviewed by Kamp et al. (*26*), macrophages and neutrophils respond to crocidolite with production of superoxide anion radicals and H_2O_2. The observation that crocidolite exposure increased H_2O_2 release by RLE-6TN cells indicates that, like macrophages and neutrophils, crocidolite can stimulate production of reactive oxygen species by rat alveolar epithelial cells.

There are several potential sources of oxidants within cells, including lipoxygenases, xanthine oxidase, cytochrome P450, and mitochondria. Regarding the latter, mitochondria are known to be sources of H_2O_2 (*27,28*). At both complex I (NADH dehydrogenase) and complex III of the respiratory chain, there can occur a single electron reduction of molecular oxygen resulting in the formation of superoxide anion radicals (*29,30*). Through either its spontaneous dismutation or the action of manganese superoxide dismutase, superoxide anion radicals in the mitochondria can be converted to H_2O_2. Our findings with TTFA, an inhibitor of electron transfer from complex II to complex III, which attenuates superoxide anion radical formation by mitochondria, indicate the mitochondrial respiratory chain is a source H_2O_2 in crocidolite-exposed rat alveolar epithelial cells. Additionally, the observation that TTFA treatment also inhibited crocidolite activation of NF-κB and MIP-2 gene expression provides evidence that mitochondrial-derived oxidants are part of a signaling pathway activated by this fiber. In this respect, the cytokine TNF-α, a potent activator of NF-κB, increases oxidant production by mitochondria (*31*). Similar to our observations, studies using the mitochondrial respiratory chain inhibitor rotenone have demonstrated that mitochondrial-derived oxidants contribute to TNF-α-induced nuclear translocation of NF-κB (*32*), suggesting there are common aspects in the signaling pathways regulating NF-κB activation in cells exposed to crocidolite and TNF-α.

In summary, here we report that crocidolite exposure of the rat alveolar type II cell line increases nuclear translocation of the transcription factor NF-κB and expression of mRNA for the neutrophil chemotactic chemokine MIP-2. Both the NF-κB and the MIP-2 responses could be attenuated by the antioxidant TMTU, indicating their dependence on oxidative stress. Exposure of RLE-6TN cells increased production of

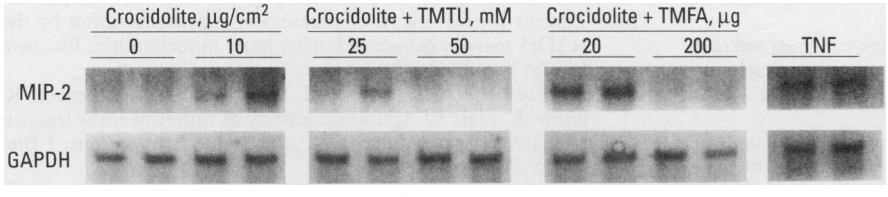

Figure 4. Crocidolite-induced increases in RLE-6TN MIP-2 gene expression are inhibited by the antioxidant TMTU and the mitochondrial respiratory chain inhibitor TTFA. Crocidolite exposure of RLE-6TN cells (10 μg/cm² × 6 hr) increased steady-state mRNA for the chemokine MIP-2, but not GAPDH. TNF-α at 50 ng/ml increased RLE-6TN MIP-2 mRNA. The addition of TMTU at 50 mM and TTFA at 200 μM attenuated crocidolite-induced increases in MIP-2 mRNA.

H_2O_2 by the epithelial cells. The source of the H_2O_2 after crocidolite exposure appears to be the mitochrondria, as treatment with the mitochondrial respiratory chain inhibitor TTFA inhibited the H_2O_2 response. Last, TTFA attenuated crocidolite activation of NF-κB and MIP-2 gene expression in the RLE-6TN cells, indicating that mitochondria-derived oxidants play a role in the signaling pathway, resulting in increased nuclear translocation of NF-κB and MIP-2 gene transcription.

REFERENCES AND NOTES

1. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of inhalation of asbestos in rats. Br J Cancer 29:252–269 (1974).
2. Mossman BT, Bignon J, Corn M, Seaton A, Gee JBL. Asbestos: scientific developments and implication for public policy. Science 247:294–301 (1990).
3. Quinlan TR, Marsh JP, Janssen YMW, Leslie KO, Hemenway D, Vacek P, Mossman BT. Dose-responsive increases in pulmonary fibrosis after inhalation of asbestos. Am J Respir Crit Care Med 150:200–206 (1994).
4. Driscoll KE, Higgins JM, Leytart MJ, Crosby LL. Differential effects of mineral dusts on the *in vitro* activation of alveolar macrophage eicosanoid and cytokine release. Toxicol in Vitro 4:284–288 (1990).
5. Driscoll KE, Hassenbein DG, Carter JM, Kunkel SL, Quinlan TR, Mossman BT. TNF and increased chemokine expression in rat lung after particle exposure. Toxicol Lett 82/83:483–489 (1995).
6. Li XY, Lamb D. and Donaldson K. The production of TNFα and IL-1-like activity by bronchoalveolar leukocytes after intratracheal instillation of crocidolite asbestos. Int J Exp Pathol 74:403–410 (1993).
7. Zhang Y, Lee TC, Guillemin B, Yu M-C, Rom WN. Enhanced IL-1β and tumor necrosis factor-α release and messenger RNA expression in macrophages from idiopathic pulmonary fibrosis or after asbestos-exposure. J Immunol 150:4188–4196 (1993).
8. Driscoll KE, Howard BW, Carter JM, Asquith TA, Detilleux P, Johnston C, Kunkel SL, Paugh D, Isfort RJ. Chemokine expression by rat lung epithelial cells: effects of *in vitro* and *in vivo* mineral dust exposure. Am J Pathol 149:1627–1637 (1996).
9. Tekamp-Olson P, Gallegos C, Bauer D, McClain J, Sherry B, Favre M, Deventer SV, Cerami A. Cloning and characterization of cDNAs for murine macrophage inflammatory protein 2, and its human homologues. J Exp Med 172:911–919 (1990).
10. Driscoll KE, Hassenbein DG, Carter J, Poynter J, Asquith TN, Grant RA, Whitten J, Purdon MP, Takigiku R. Macrophage inflammatory proteins 1 and 2: expression by rat alveolar macrophages, fibroblasts, and epithelial cells and in rat lung after mineral dust exposure. Am Rev Respir Cell Mol Biol 8:311–318 (1993).
11. Rosenthal GJ, Germolec DR, Blazka ME, Corsini E, Simeonova P, Pollock P, Kong L-Y, Kwon J, Luster MI. Asbestos stimulates IL-8 production from human epithelial cells. J Immunol 153:3237–3244 (1994).
12. Widmer U, Manoque KR, Cerami A, Sherry B. Genomic Cloning of promoter analysis of macrophage inflammatory protein (MIP)-2, MIP-1α, and MIP-1β, members of the chemokine superfamily of proinflammatory cytokines J Immunol 150:4996–5012 (1993).
13. Schreck R, Rieber P and Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in activation of NΦκB transcription facto and HIV. EMBO J 10:2247–2258 (1991).
14. Driscoll KE, Iype PT, Kumari HL, Carter JM, Aardema MJ, Crosby LL, Chestnut MH and LeBoeuf RA Establishment of immortalized alveolar type II cell lines from adult rat lungs. In Vitro Cell Develop Biol 31:516–527 (1995).
15. Janssen YMW, Driscoll KE, Howard BW, Quinlan T, Treadwell M, Barchowsky A, Mossman BT. Asbestos causes translocation of p65 protein and increases NF-κB DNA binding activity in rat lung epithelial and pleural mesothelial cells. Am J Pathol 151: 89–401 (1997).
16. Janssen YMW, Barschowsky A, Treadwell M, Driscoll KE, Mossman BT. Asbestos induces NFκB binding activity and NFκB dependent gene expression in tracheal epithelial cells. Proc Natl Acad Sci USA92:8458–8462 (1995).
17. Staal F, Roederer M, Herzenberg LA. Intracellular thiols regulate activation of NFκB and transcription of HIV. Proc Natl Acad Sci USA 87:9943–9947 (1990).
18. Driscoll KE, Hassenbein DG, Howard BW, Isfort RJ, Cody D, Tindal MH, Carter JM. Cloning, expression, and functional characterization of rat macrophage inflammatory protein. 2: A neutrophil chemoattractant and epithelial cell mitogen. J Leuk Biol 58:359–364 1995.
19. Fort P, Marty L., Piechaczyk, Sabrouty SE, Dani C, Jeanteur P, Blanchard JM. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate dehydrogenase multigenic family. Nucl Acid Res 13:1431–42 (1985).
20. Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitorgen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. J Immunol 141:2407–2412 (1988).
21. Zar JH. Biostatistical Analysis, 2nd ed. Englewood Cliffs, NJ: Prentice-Hall, 1984.
22. Baeuerle PA, Henkel T. Function and activation of NFκB in the immune system. Ann Rev Immunol 12:141–180 (1994).
23. Simeonova PP, Luster MI. Asbestos induction of nuclear transcription factors and interleukin-8 gene regulation. Am J Respir Cell Mol Biol 15:787–795(1996).
24. Zhao Q, Simpson L, Driscoll KE, Leikauf GD. Chemokine regulation of ozone-induced neutrophil and monocyte inflammation. Am J Physiol Lung Cell Mol Biol 18:L39–L46 (1998).
25. Lund LG, Aust AE Iron-catalyzed reactions may be responsible for the biochemical and biological effects of asbestos. Biofactors 3:83–89 (1991).
26. Kamp DW, Graceffa P, Pryor WA, Weitzman SA The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 12:293–315, (1992).
27. Boveris A, Cadenas E, Stoppani AOM. Role of ubiquinone in the mitochondrial generation of hydrogen peroxide. Biochem J 156:435–444 (1976).
28. Forman HJ, Boveris A. Superoxide and hydrogen peroxide in mitochondria. In: Free Radicals in Biology, Vol 5. New York:Academic Press, 1982;65–90.
29. Turrens JF, Alexandre A, Lehninger AL. Ubisemiquinone is the elctron donor for superoxide formation by complex III of heart mitochondria. Arch Biochem Biophys 237:408–411 (1985)
30. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427 (1980).
31. Schulze-Ostoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob W, Fiers W. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. J Biol Chem 267:5317–5323 (1992).
32. Schulze-Ostoff K, Beyaert R, Vandevoorde V, Haegeman G, Fiers W. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO 12:3095–3104 (1993).